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Cell-free Protein Synthesis directed by Coliphage MS2 RNA: Synthesis of Intact Viral Coat Protein and Other Products

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Coliphage MS2 RNA directs the synthesis in *Escherichia coli* extracts of a protein product the tryptic peptides of which correspond to the peptides of the phage coat protein. Fingerprint analysis of the synthetic protein labeled with various [^{14}C]amino acids indicated that in each case only those coat protein peptides which contain a particular amino acid were labeled. The formation of whole coat protein molecules as the predominant product was shown by co-chromatography on Sephadex with authentic phage coat subunit. Sequential synthesis of coat protein ending at the carboxyl end was observed by pulse-labeling with radioactive amino acid and by analysis of the peptides formed in the presence of puromycin. In addition to phage coat protein, MS2 RNA directed the synthesis of other proteins. These could be preferentially labeled with histidine, an amino acid lacking in the coat protein, and partially separated on Sephadex.

1. Introduction

Infection of a cell by an RNA virus leads to the synthesis of at least two new proteins, an RNA-dependent RNA polymerase (Baltimore & Franklin, 1962; Weissman, Simon & Ochoa, 1963; August, Cooper, Shapiro & Zinder, 1963; Haruna, Nozu, Ohtaka & Spiegelman, 1963) and the coat protein of the virus. Certain aspects of this process can be studied in cell extracts in which viral RNA serves as template for protein synthesis (Nirenberg & Matthaei, 1961). Among the proteins formed in the *Escherichia coli* cell-free system with coliphage f2 RNA as messenger is a product the tryptic peptides of which correspond to those of the coat protein of the phage (Nathans, Notani, Schwartz & Zinder, 1962). Other proteins are made also, since histidine, an amino acid lacking in the coat proteins of coliphages f2 (Nathans *et al.*, 1962) and MS2 (Ohtaka & Spiegelman, 1963), is incorporated into protein when phage RNA is present (Nathans *et al.*, 1962; Ohtaka & Spiegelman, 1963; Valentine & Zinder, 1963). In this paper we report on further characterization of the protein products the synthesis of which is directed by coliphage RNA. The results indicate that the major protein consists of intact subunits of the phage coat; in addition, other minor products have been detected.

2. Materials and Methods

(a) *Cell extracts*

Extracts of *E. coli* B, grown as previously described (Nathans & Lipmann, 1961) and frozen as cell paste, were prepared according to the method of Nirenberg & Matthaei (1961) as described elsewhere (Nathans *et al.*, 1962). The only change from our previous procedure

was in the pre-incubation of the S30† fraction, which was carried out in 0·01 M-tris–HCl (pH 7·4), 0·01 M-magnesium acetate, 0·05 M-KCl and 0·005 M-mercaptoethanol at 35°C for 45 min. Protein synthesis was carried out at 35°C in volumes varying from 0·04 to 1 ml. containing 0·003 M-ATP, 0·01 M-phosphoenolpyruvate, 30 μg/ml. pyruvate kinase, 0·0001 M-GTP, 0·01 M-glutathione, 0·05 M-KCl, 0·015 M-magnesium acetate, 0·05 M-tris–HCl (pH 7·8), 0·5 mg/ml. *E. coli* sRNA, 2×10^{-5} mole of each [^{12}C]amino acid minus the radioactive one, 2×10^{-6} mole of a [^{14}C]- or [^{3}H]amino acid, 1/4 vol. of pre-incubated S30, and approximately 0·5 mg/ml. MS2 RNA. [^{14}C]Protein synthesized in the cell-free system was measured by spotting a portion of the reaction mixture (usually 0·025 ml.) on a Whatman 3 MM filter paper disc to which a drop of [^{12}C]amino acid had been applied. The discs were then washed and counted as described by Mans & Novelli (1961).

(b) *MS2 RNA and protein*

MS2 and its host *E. coli* C3000 were obtained from R. L. Sinsheimer. The growth and purification of MS2 and preparation of phage RNA have been described elsewhere (Shimura, Moses & Nathans, 1965). Coat protein was recovered from the phenol phase after preparation of phage RNA by extracting with ether and washing the precipitated protein with 5% TCA, alcohol–ether 1:1 (v/v) and ether.

[^{3}H]Tyrosine-labeled MS2 was prepared by growing the phage in 100 ml. of glutamate-enriched salts medium (Shimura *et al.*, 1965) in the presence of 120 μc [^{3}H]tyrosine (430 μc/μmole). The purified radioactive phage gave $8{\cdot}9 \times 10^{3}$ cts/min per A_{260} unit.

(c) *Fractionation of proteins on Sephadex*

In order to co-fractionate the coat protein of MS2 and the proteins made in the cell-free system, the subunits of the phage coat were first dissociated in guanidine. For this purpose a mixture of purified MS2 labeled with [^{3}H]tyrosine and the synthetic product labeled with [^{14}C]amino acid was incubated at 45°C for 6 hr in 6·5 M-guanidine hydrochloride–0·05 M-tris hydrochloride at pH 8·5. The sample was then applied to a 1 cm × 25 cm column of G200 Sephadex (40 to 120 μ particle size) which was equilibrated with the guanidine–tris solution at 35°C. Proteins were then eluted with guanidine–tris at 35°C and the effluent fractions analyzed for [^{14}C]- and [^{3}H]protein by precipitation with 5% TCA in the presence of carrier protein and excess [^{12}C]amino acids. After extraction of the precipitate with 5% TCA at 90°C for 15 min and further washing with TCA and alcohol–ether, the samples were dissolved in hyamine and counted.

(d) *Fingerprinting*

To prepare newly synthesized, radioactive protein for fingerprinting, excess non-radioactive amino acid was added to the incubation mixture, followed by TCA to give 5% concentration. The precipitate was extracted with 5% TCA at 90°C for 15 min and washed 4 times with TCA, twice with alcohol–ether (1:1) and once with ether. To the dried product, 5 mg of coat protein was added and the mixture oxidized with performic acid. For this purpose 10 ml. 99% formic acid was allowed to stand with 0·5 ml. of 30% hydrogen peroxide at room temperature for 1 hr. To an estimated 10 mg of protein, 0·5 ml. of oxidizing reagent was added. After 20 min at room temperature, excess water was added and the solution lyophilized. The lyophilized protein was then dissolved at pH 10 to 11 with dilute KOH and the solution quickly brought to pH 8·0. Digestion with twice crystallized trypsin in an amount approximately equal to 1% of the weight of protein was carried out in a pH stat at pH 8·0. At the end of the reaction the sample was dried in a desiccator.

Separation of tryptic peptides was performed by procedures similar to those described by Dintzis (1961) for hemoglobin. The dried peptides were taken up in a small volume of buffer and applied to an 18 in. × 22 in. Whatmann 3 MM chromatography paper for electrophoresis on a water-cooled plate. The buffer was pyridine–acetate of pH 4·5 (1·2% pyridine, 1·3% glacial acetic acid, 2·5% *n*-butanol, v/v in water). After 2½ hr at 30 v/cm,

† Abbreviations used: S30, 30,000 *g* supernatant of alumina-ground *E. coli*; TCA, trichloracetic acid.

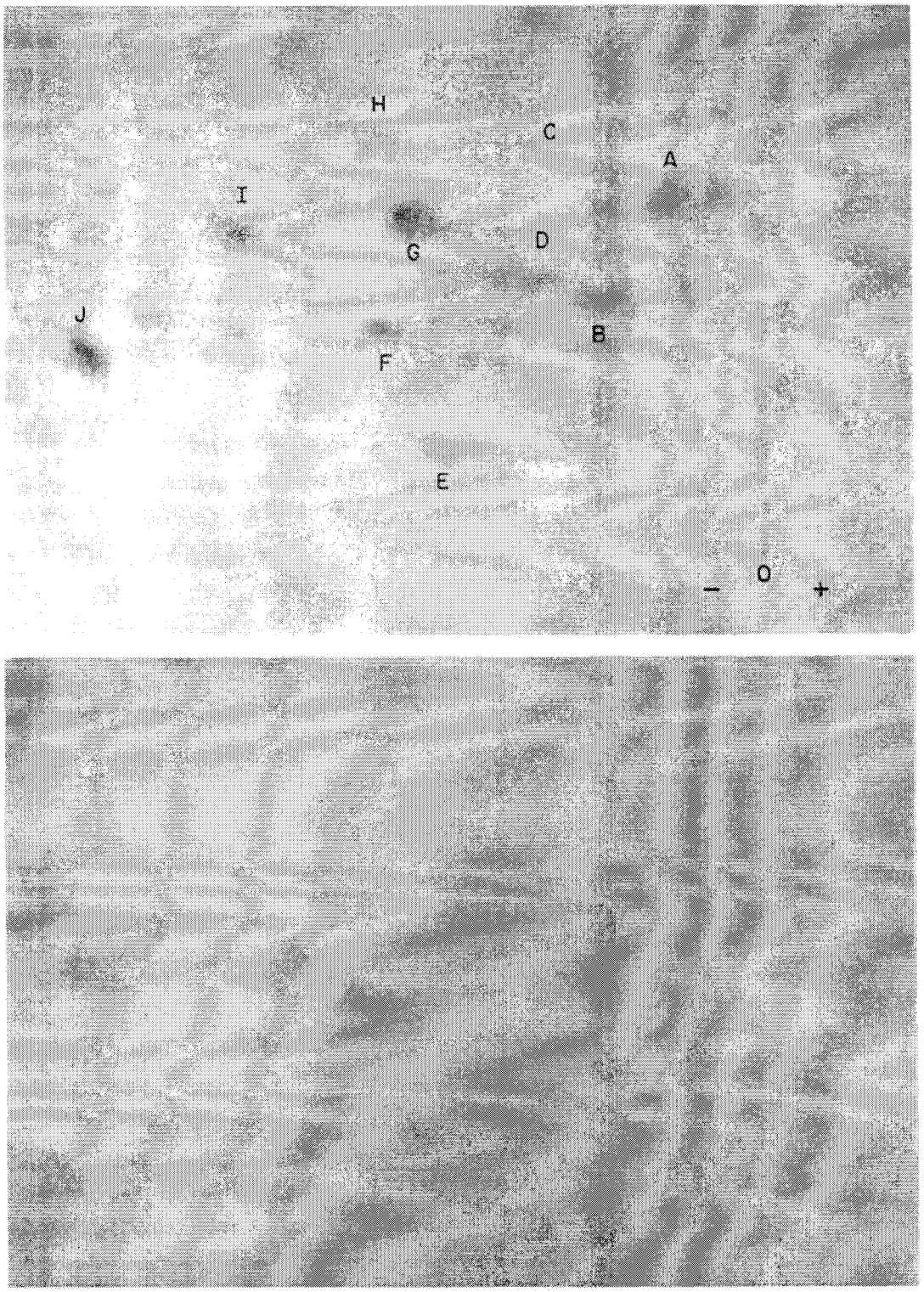

PLATE I. Fingerprint and radioautogram of [^{14}C]alanine-labeled product. Protein synthesis was carried out as described in Materials and Methods for 40 min in the presence of [^{14}C]alanine and 0·3 mg MS2 RNA in 0·5 ml. The protein of the 105,000 *g* supernatant fraction of the incubation mixture containing 40,000 cts/min was fingerprinted with carrier coat protein as described in Materials and Methods. Above is the ninhydrin-stained paper; below is the corresponding radioautogram, developed after 1 week. The circled spot on the fingerprint denotes a peptide unstained by ninhydrin, but detectable by fluorescence with ultraviolet light. Peptide H, which is produced in variable yield, did not appear in this radioautogram, but was clearly seen in other runs.

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the paper was dried and chromatographed overnight in the other dimension in *n*-butanol–acetic acid–pyridine–water (75:15:50:60). To delineate [^{14}C]peptides a radioautogram was prepared by applying the dried paper to X-ray film for 1 to 2 weeks. The paper was then dipped in an acetone solution of ninhydrin (0·25%) and the peptides of the carrier coat protein compared to the spots on the X-ray film. The peptide spots were cut out, eluted with water in counting vials, and the eluted peptides dried at 80°C overnight. Hyamine was added to each vial and radioactivity measured in a liquid-scintillation counter using 2,5-diphenyloxazole and 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene in toluene. In instances where the ^{3}H/^{14}C ratio of peptides was determined, duplicate runs showed a maximum variation of 20% in this ratio.

(e) *Chemicals*

[^{14}C]Amino acids were obtained from New England Nuclear Corp., Boston, Mass., and had specific activities of approximately 30 mc/milliatom of carbon. [^{3}H]Amino acids were from Nuclear Chicago Corp., La Grange, Ill.; [^{3}H]alanine had a specific activity of 1·1 c/m-mole. Puromycin was a gift of Dr Leon Goldman of the Lederle Laboratories, Pearl River, N.Y. *E. coli* soluble RNA was purchased from General Biochemicals, Chagrin Falls, Ohio; and Sephadex G200, and Blue Dextran from Pharmacia Fine Chemicals, Inc., New York, N.Y. Trypsin, *E. coli* alkaline phosphatase and egg white lysozyme were obtained from Worthington Biochemical Corp., Freehold, N.J.

3. Results

(a) *Coat protein synthesis*

It had been shown previously that one of the proteins synthesized in *E. coli* extracts in the presence of f2 RNA is identifiable as the coat protein of the phage on the basis of correspondence of electrophoretic mobilities of the tryptic peptides of the coat protein with those of the radioactively labeled synthetic product (Nathans *et al.*, 1962). Similar observations have been made with the RNA of the related coliphage MS2. When the synthetic product labeled with various [^{14}C]amino acids was fingerprinted together with MS2 coat protein and the distribution of radioactive peptides determined by radioautography, the results shown in Plate I and Table 1 were obtained. As seen in the Plate, when [^{14}C]alanine was the label, ninhydrin-stained peptides corresponded with radioactive spots appearing on the radioautogram. Similar results were obtained with various radioactive amino acids as precursors, and in each case only those peptides which contained the particular amino acid had corresponding radioactive spots (Table 1).

The fingerprint shown in Plate I came from an analysis of the 105,000 *g* supernatant fraction of the incubation mixture, which accounts for 15 to 30% of the total labeled protein. Similar results were obtained when the ribosome-bound product was analyzed. That this fraction consists largely of coat protein chains identical to those in the supernatant fraction is shown by analysis of a mixture of ribosome-bound product labeled with [^{14}C]alanine and soluble product labeled with [^{3}H]alanine. As seen in Table 2, the relative amount of each tryptic peptide of the coat protein was the same in both fractions. Since the bulk of the radioactivity in the fingerprint of soluble as well as ribosome-bound protein corresponds to coat protein peptides, it appears that the predominant product made in the cell-free system is the coat protein (also see below). A similar conclusion was reached by Ohtaka & Spiegelman (1963) on the basis of electrophoretic separation of the products.

TABLE 1

Incorporation of amino acids into coat protein peptides

Peptide	Composition				Incorporation			
	Lys	Arg	Ala	Tyr	Lys	Arg	Ala	Tyr
A	−	−	+	+	−	−	+	+
C	−	+	+	−	−	+	+	−
E	−	+	+	−	−	+	+	−
F	+	−	+	+	+	−	+	+
G	+	−	+	−	+	−	+	−
H	+	−	+	−	+	−	+	−
I	+	−	−	+	+	−	−	+
J	+	−	−	−	+	−	−	−

[^{14}C]Amino acid-labeled product was fingerprinted with carrier coat protein as described in Materials and Methods and the ninhydrin-stained peptides compared with the radioautogram spots. Under composition, + indicates the presence of an amino acid in the particular peptide and − its absence. Under incorporation, + indicates presence of a spot on the radioautogram corresponding to the stained peptide and − the absence of such a spot. Peptides B and D are usually not clearly seen on the fingerprint or radioautogram. The data on amino acid composition are from M. Naughton (personal communication).

TABLE 2

Relative amount of tryptic peptides in ribosome-bound product and soluble product

Peptide	Relative amount (R/S) ^{14}C/^{3}H	
	Exp. 1	Exp. 2
A	1·0	1·0
C	1·1	1·0
E	1·0	1·0
F	1·0	1·1
G	1·0	1·2
H	1·2	1·1
O	1·1	0·90

Protein was synthesized in one tube with [^{14}C]alanine, and in another tube with [^{3}H]alanine. The ribosomes were sedimented at 105,000 *g* for 2 hr and washed once with 0·01 M-tris (pH 7·4)–0·01 M-magnesium acetate. [^{14}C]Ribosome fraction (about 100,000 cts/min in protein) was mixed with [^{3}H]supernatant fraction (about 200,000 cts/min in protein) and the mixture fingerprinted as described in Materials and Methods. Each alanine-containing peptide was eluted and counted. In each experiment, the carboxyl-terminal peptide, A (M. Naughton, personal communication) was assigned a ^{14}C/^{3}H ratio of 1·0, and the others normalized to this value. Actual counts varied from 200 to 7600 cts/min. R, ribosomal product; S, soluble product.

(b) *Evidence for synthesis of whole molecules of coat protein*

Although it is clear that all parts of the coat protein molecule detectable by the fingerprinting technique are synthesized in cell extracts, the available evidence does not exclude the possibility that a large part of the product consists of a series of coat protein fragments. Such fragments could arise, for example, by initiation of synthesis at various points and/or premature termination of growing polypeptide chains. If fragments of the coat protein were major products, one might expect grossly different amounts of the various tryptic peptides obtained from the synthetic product. Analysis of the relative amount of each of three tyrosine-containing tryptic peptides present in the product indicates, however, that two were formed in equal quantity, and the third (the carboxyl terminal peptide) was formed in somewhat reduced amount (Table 3). This result is consistent with the synthesis primarily of complete coat protein molecules.

TABLE 3

Relative amounts of tyrosine peptides

Peptide	Relative amount $^{14}C/^{3}H$
A	1·0
F	1·5
I	1·6

[^{14}C]Tyrosine-labeled product from the supernatant fraction of the incubation mixture (56,000 cts/min in protein) was added to [^{3}H]tyrosine-labeled MS2 (135,000 cts/min) and the mixture fingerprinted as described in Materials and Methods. The three tyrosine-containing peptides were eluted and counted. The carboxyl-terminal peptide (A) was assigned a $^{14}C/^{3}H$ ratio of 1·0, and the others normalized to this value. Actual counts varied from 205 to 2390 cts/min.

More direct evidence for the formation of intact coat protein comes from fractionation of the cell-free product on Sephadex. As shown in Fig. 1, the coat protein of MS2, when dissociated by treating the phage with 6·5 M-guanidine, as described in Materials and Methods, is eluted from G200 Sephadex in a single peak between *E. coli* alkaline phosphatase and egg white lysozyme. (After performic acid oxidation, the coat protein is eluted at the same effluent volume. The apparent molecular weight of the coat protein subunit estimated by gel filtration is approximately 24,000.) Sephadex fractionation can therefore be used to examine the product of the cell-free system for intact coat protein molecules. When [^{14}C]threonine-labeled product of the cell-free system was mixed with [^{3}H]tyrosine-labeled MS2 and the mixture treated with 6·5 M-guanidine, fractionation on G200 Sephadex in the presence of guanidine showed that about 70% of the [^{14}C]protein corresponded to intact coat protein subunit (Fig. 2). As seen in the Figure, other protein products, which are eluted prior to the coat protein, are also present (see below). The fact that little or no [^{14}C]protein is eluted after the dissociated coat protein indicates that fragments of coat protein large enough to be TCA-insoluble are not synthesized at an appreciable rate in the cell-free system. This evidence thus indicates that whole coat protein molecules constitute the major product in the cell-free system.

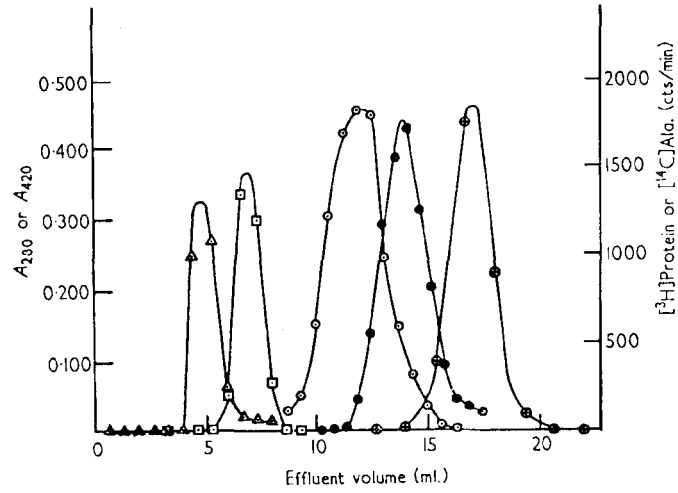

FIG. 1. Properties of G200 Sephadex column. The Figure is a composite of three consecutive runs on the same column. Conditions for chromatography are given in Materials and Methods. —△—△—, Blue Dextran (average molecular weight 2×10^6); —□—□—, *E. coli* alkaline phosphatase; —○—○—, dissociated MS2 coat protein; —●—●—, egg white lysozyme; —⊕—⊕—, [^{14}C]alanine. Blue dextran and lysozyme were measured at 280 mμ. Alkaline phosphatase was assayed on dialyzed fractions, which gradually regain activity, using *p*-nitrophenylphosphate as substrate and measuring the change in A_{420}; the plot of activity is the change in A_{420} per 10 min at pH 8·5 and 23°C. Coat protein was labeled with [^{3}H]tyrosine and counted as described in Materials and Methods. [^{14}C]Alanine was counted on filter paper discs.

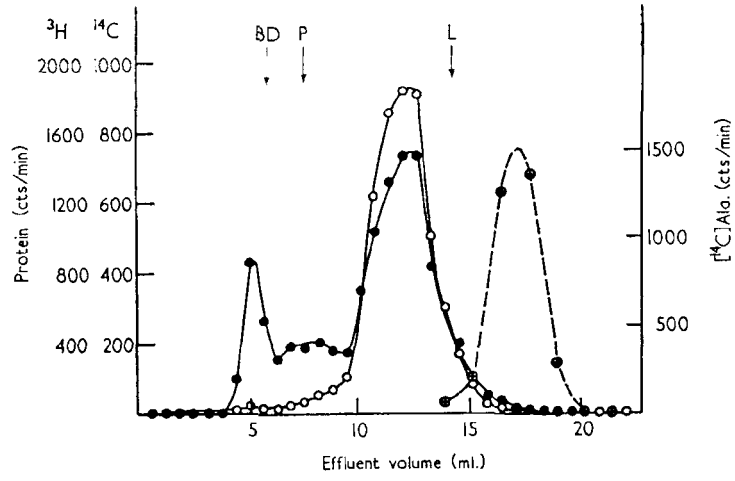

FIG. 2. Sephadex chromatography of [^{14}C]threonine-labeled product together with [^{3}H]tyrosine-labeled MS2 coat protein. Conditions for chromatography are given in Materials and Methods. The ^{14}C-product was the unwashed ribosome fraction of the incubation mixture, which contained 85% of the total protein counts. Recovery of input acid-insoluble radioactive material was 96% for ^{3}H and 94% for ^{14}C. —○—○—, [^{3}H]Protein; —●—●—, [^{14}C]protein; —⊕—⊕—, [^{14}C]alanine, added as a marker. The arrows indicate elution volumes for Blue Dextran (BD), phosphatase (P) and lysozyme (L).

(c) *Sequential synthesis of coat protein*

If coat protein synthesis begins from a fixed starting point and proceeds toward the carboxyl end (Bishop, Leahy & Schweet, 1960; Dintzis, 1961; Goldstein, Goldstein & Lowney, 1964) one would expect differential labeling of coat protein peptides after a brief initial pulse of a radioactive amino acid. To test this expectation, pulse-labeled protein was prepared by incubating the cell-free system for 5 to 10 minutes with [^{3}H]alanine followed by further 35 or 30-minute incubation, respectively, in the presence of excess non-radioactive alanine. As a reference, protein was also made in the presence of [^{14}C]alanine during a 40-minute incubation. The pulse-labeled product ([^{3}H]alanine) was then mixed with the reference product ([^{14}C]alanine), carrier coat protein added, the mixture fingerprinted as described in Materials and Methods, and the eluted peptides counted. This type of pulse should result in a gradient of labeling of the synthesized protein, highest at the amino end and lowest at the carboxyl end, i.e., the ^{3}H/^{14}C ratio, which is a measure of the relative amount of peptide formed during the pulse, should decrease from amino to carboxyl end of the polypeptide chain. As seen in Fig. 3, a gradient of labeling was achieved amounting to a five-fold difference between the peptide(s) formed in greatest amount (the tryptic core) and the two formed in least amount, one of which (A of Fig. 3) is the carboxyl terminal peptide. As a control, a product made during a 40-minute incubation with [^{3}H]alanine showed no gradient. (Similar pulse-labeling experiments have been carried out by G. von

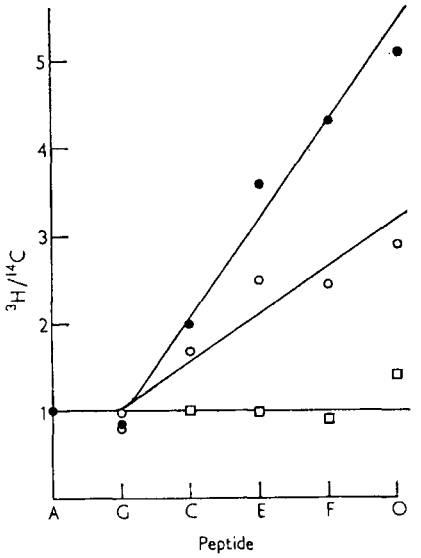

FIG. 3. Sequential synthesis of coat protein. Pulse-labeled protein containing [^{3}H]alanine (about 150,000 cts/min) was prepared as described in the text and mixed with [^{14}C]alanine product (about 100,000 cts/min) made during prolonged incubation. The labeled tryptic peptides of the coat protein were isolated and counted as described in Materials and Methods. The Figure shows the relative amount (^{3}H/^{14}C ratio) of each alanine peptide made during an initial 5-min (—●—●—) or 10-min pulse (—○—○—) compared with the 40-min control (—□—□—). In each case peptide A, the C-terminal peptide, was assigned a ratio of 1·0 and the others normalized to this value. Actual counts varied from 200 to 2400 cts/min. The yield of peptide H, which also contains alanine, was too low for accurate determination of the ^{3}H/^{14}C ratio.

Ehrenstein (personal communication).) Although it is not as yet possible to relate the relative amount of each peptide formed to its chemically determined position in the protein, the fact that there is a consistent gradient with the carboxyl-terminal peptide made in least amount strengthens the case for sequential synthesis of the whole coat protein molecule starting at the amino end.

(d) *Effect of puromycin on coat protein synthesis*

The sequential formation of coat protein can also be examined with the use of the antibiotic puromycin, which has been shown to interrupt growth of the polypeptide chain (Morris, Arlinghaus, Flavelukes & Schweet, 1963; Allen & Zamecnik, 1962; Gilbert, 1963; Nathans, Allende, Conway, Spyrides & Lipmann, 1963). By determining the amount of each peptide formed in the presence of puromycin relative to the amount formed in the uninhibited system, it is possible to determine the sequence of coat protein synthesis, as shown in the model of Fig. 4. For this purpose, coat protein

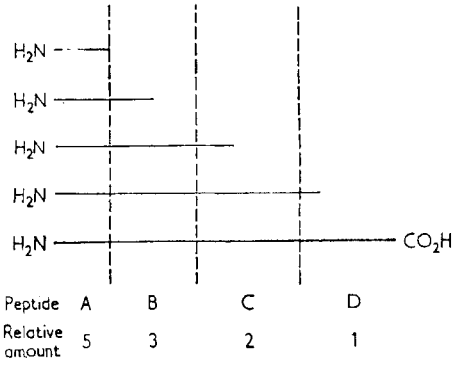

FIG. 4. Model of coat protein fragments expected in presence of puromycin. Vertical lines indicate points of cleavage by trypsin.

labeled with [^{3}H]alanine was made in the presence of sufficient puromycin to inhibit incorporation by 71%, and as a reference, coat protein labeled with [^{14}C]alanine was prepared in a parallel reaction tube in the absence of puromycin. The 105,000 *g* supernatant fractions of these samples were then mixed in appropriate amounts, carrier coat protein added, and the mixture analyzed by fingerprinting, as described in Materials and Methods. The ^{3}H/^{14}C ratio of a tryptic peptide is a measure of the relative amount of that peptide made in the presence of puromycin, which in turn reflects the sequence of synthesis of the coat protein molecule (Fig. 4). As shown in Fig. 5, a gradient of labeling of the coat protein peptides is evident in the presence of puromycin, and the sequence of synthesis of the protein determined in this way corresponds rather well to that determined by means of pulse-labeling (Fig. 3). (Included in Fig. 5 are the results of two control experiments in which chloramphenicol or tetracycline instead of puromycin was used to inhibit protein synthesis. As seen in the Figure, neither of these antibiotics led to a gradient of labeling.)

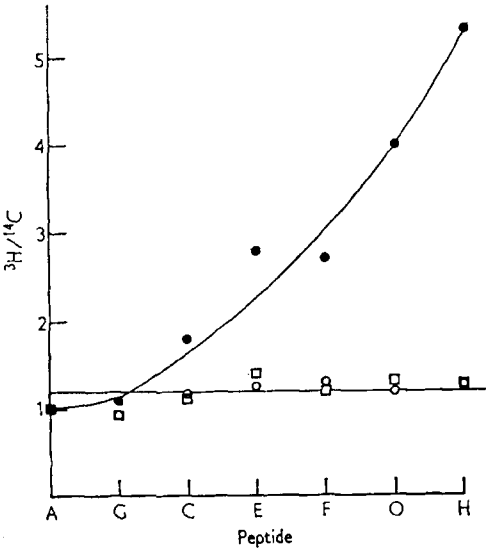

FIG. 5. Differential synthesis of coat protein peptides in the presence of puromycin. Protein labeled with [^{3}H]alanine was synthesized in the presence of $1{\cdot}5 \times 10^{-6}$ M-puromycin during a 40-min incubation, and in a second tube protein labeled with [^{14}C]alanine was synthesized in the absence of puromycin, as described in the text. The 105,000 *g* supernatant fractions of the two samples were mixed (200,000 cts/min ^{3}H and 100,000 cts/min ^{14}C, measured in the hot TCA precipitate) and fingerprinted as described in Materials and Methods. In parallel experiments, samples of [^{3}H]alanine-labeled product prepared in the presence of chloramphenicol (3·5 μg/ml., 64% inhibition) or tetracycline (4×10^{-5} mole, 77% inhibition) were mixed with [^{14}C]alanine product and fingerprinted. The relative amount of each alanine peptide formed in the presence of inhibitor (^{3}H/^{14}C ratio) is plotted. In each case the C-terminal peptide (A) has been assigned a ratio of 1·0 and the others normalized to this value. The order of peptides along the abscissa is the same as that in Fig. 3 to facilitate comparison of the Figures. —●—●—, Puromycin; —○—○—, chloramphenicol; —□—□—, tetracycline.

(e) *Synthesis of other protein(s)*

Since the coat proteins of coliphages f2 and MS2 lack histidine, incorporation of this amino acid into protein in the presence of phage RNA has been taken to indicate that one or more proteins other than that of the virus coat is synthesized in the cell-free system (Nathans *et al.*, 1962; Ohtaka & Spiegelman, 1963; Valentine & Zinder, 1963). Incorporation of histidine is stimulated 20- to 40-fold by phage RNA, though the total incorporation is only about 1/5 to 1/20 that of several other amino acids (leucine, arginine, tyrosine, proline, threonine, isoleucine, lysine). Ohtaka & Spiegelman (1963) have shown that the histidine-containing proteins are electrophoretically distinct from the phage coat protein. We have analyzed the histidine product by gel filtration as described above. As shown in Fig. 6, when the ^{14}C-product is mixed with [^{3}H]MS2, treated with guanidine and passed through Sephadex, the [^{14}C]protein in the effluent does not correspond with the [^{3}H]carrier phage coat protein. Instead, several histidine-containing protein peaks are apparent, one of which comes through at the void volume. These results indicate that little or no histidine is being incorporated into phage coat (due to errors of the cell-free system), but rather that proteins distinct from the phage coat are formed.

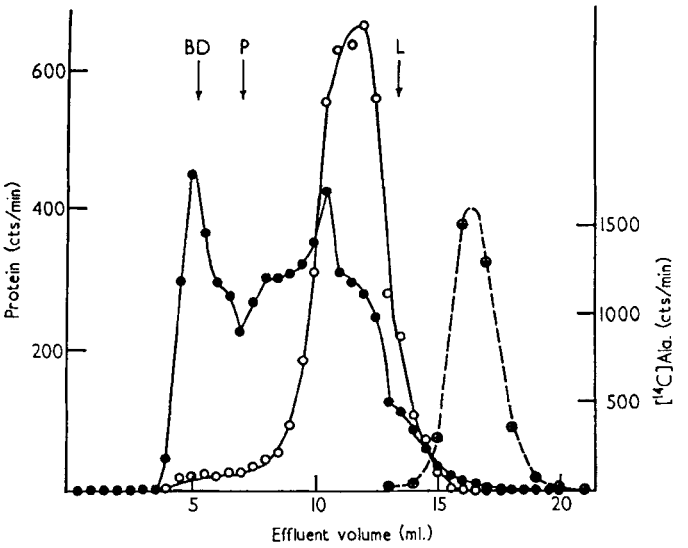

FIG. 6. Sephadex chromatography of [^{14}C]histidine-labeled product together with [^{3}H]tyrosine-labeled MS2 coat protein. Conditions for chromatography are given in Materials and Methods. The [^{14}C]product was the unwashed ribosome fraction of the incubation mixture, which contained 90% of the total radioactive protein. Recovery of input acid-insoluble radioactive material was 93% for ^{3}H and 96% for ^{14}C. —○—○—, [^{3}H]Protein; —●—●—, [^{14}C]protein; —⊕—⊕—, [^{14}C]alanine, added as a marker.

4. Discussion

The biosynthesis of coliphage coat protein in the presence of phage RNA is the only presently available cell-free system in which pure messenger RNA directs the synthesis of a specific identifiable protein. In the present paper I have examined two aspects of this system: (1) whether whole coat protein molecules are made, and (2) the synthesis of proteins other than phage coat.

The most direct evidence which points to the synthesis of whole coat protein molecules is the co-chromatography on Sephadex of the synthetic product and authentic coat protein under conditions where aggregation is prevented by guanidine. Less direct evidence which points to the same conclusion are the observations of Ohtaka & Spiegelman (1963) on co-electrophoresis of synthetic product with phage coat, the sequential synthesis of different parts of the coat protein, ending at the carboxyl end of the molecule, and the nearly equivalent amounts of the three peptides which contain tyrosine, one of which is the C-terminal peptide.

The finding of intact coat protein molecules as the major product in the rather crude S30 system is somewhat surprising. It is known that such extracts contain abundant ribonuclease activity, resulting in the rapid degradation of added virus RNA (Barondes & Nirenberg, 1962; Nathans, unpublished observations). Therefore, one might expect to find a large number of coat protein fragments made on partially degraded templates. The failure to detect such fragments, on the other hand, suggests that only complete templates function in this system, at least at an appreciable rate. It is possible that this discrimination occurs at the level of messenger RNA attachment to ribosomes or perhaps in the form of required releasing signals. Fragments of RNA unable to become attached to ribosomes would be totally inactive, whereas fragments with no

releasing codon would form incomplete peptide chains, which, however, would remain bound to the template and prevent consecutive cycles of protein synthesis on that template.

Protein products other than the virus coat have been detected in the cell-free system by means of histidine incorporation, since this amino acid is lacking in the coat protein. Chromatography of the histidine-containing product on Sephadex has revealed considerable heterogeneity, including components of larger size than the coat protein subunit. Whether the smaller components are breakdown products of the larger one is not known; nor is it known whether the larger components consist of smaller polypeptide chains joined by disulfide linkages formed during preparation of the sample. Although MS2 RNA is of sufficient size to code for several proteins (Strauss & Sinsheimer, 1963), only two virus-determined proteins have so far been detected in the infected cell, an RNA-dependent RNA polymerase (Weissman *et al.*, 1963; August *et al.*, 1963; Haruna *et al.*, 1963) and the coat protein. Whether any of the histidine-containing proteins made in cell extracts is the polymerase is yet to be determined.

The predominance of phage coat over the other protein products is unexplained. The possibility that it reflects control mechanisms which operate in the phage-infected cell has already been raised (Ohtaka & Spiegelman, 1963). On the other hand, this difference in protein yields may be an artifact of the cell-free system due, for example, to greater susceptibility of larger or less ordered cistrons to ribonuclease present in the extract.

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